RESEARCH NOTE

COMPOSITION STUDIES ON TOBACCO. XLII. SMOKE CONDENSATE NEUTRALS STRONGLY ADSORBED DURING COLUMN CHROMATOGRAPHY

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Recent studies in this laboratory have involved the distribution of biological activity after fractionating cigarette smoke condensate. In this work, the neutrals were separated by adsorption chromatography on activated silicic acid followed by solvent partitioning of the eluates (4,5). Although biologically active fractions were obtained by this method, severe losses in activity were observed when all eluates were combined to obtain a reconstituted neutral fraction (3). In addition to other factors, it appeared that such losses may have been due in part to a failure to elute the columns completely. Even after extensive elution with methanol, all columns showed a tan color, indicating that some material remained thereon. Other workers have also observed this effect (6,7) and inspection of their data showed that up to 18 per cent of the neutral fraction was not eluted.

To study this problem further, activated and nonactivated silicic acid and Florisil were employed to separate the neutrals of cigarette smoke condensate by the previously described method (5). "Nonactivated" adsorbents refer here to the use of the material as received from the supplier; under these conditions silicic acid and Florisil contained 5 and 6 per cent water, respectively. Activated adsorbents were prepared by heating at 150°C for 17 hrs. The results obtained are shown in **Table 1.** The activated adsorbents retained much more material than the nonactivated, and more material could be eluted from silicic acid than from Florisil. Further amounts of the retained neutrals could be removed from all adsorbents by elution with pyridine either *in situ* or after removal of the adsorbent from the column and slurrying extensively with this solvent. Sequential treatment of the silicic acid with acetic acid was more effective than initial treatment with pyridine. Because acetic acid partially dissolved the Florisil, an evaluation of this solvent could not be made with this system. A maximum removal of 99.7 per cent was achieved with nonactivated silicic acid, but the column after elution still retained colored material by visual inspection.

In adding the neutral fraction to chromatographic columns, a solution of 5 per cent benzene in petroleum ether was required since the material was not completely soluble in the first eluting solvent, i.e. petroleum ether. Subsequent addition of the latter to initiate the elution resulted in precipitation on top of the adsorbent of a finely divided black material that resembled the strongly adsorbed substances. As the solvent polarity was increased, this material ultimately dissolved and entered the adsorbent. To determine whether this precipitate contained the strongly adsorbed substances, the neutral fraction was suspended in petroleum ether and the insoluble material was filtered off. The filtrate and precipitate (1.3 per cent of the total neutrals) were then independently chromatographed on activated silicic acid. Although ether and methanol eluted proportionately more weight from the column containing the precipitate, strongly adsorbed material still remained on the columns after elution with methanol in both instances. However, the amount of such material appeared to be greater for the petroleum ether-insoluble fraction.

In another experiment dealing with this problem, the neutrals were chromatographed on activated silicic acid, and the material eluted with methanol was partitioned between petroleum ether and 90 per cent aqueous methanol as previously described (5). The aqueous methanol-soluble fraction was then rechromatographed on neutral alumina of low activity (1). Elution with the above series of solvents resulted in removal of only 65 per cent of the added material. The appearance of the column after elution with methanol resembled that of silicic acid after chromatography of the original neutral fraction.

None of these findings definitively show that the strongly adsorbed material is either a true neutral component of condensate or an artifact produced during chromatography. However, the results on alumina may indicate that the latter is occurring to some extent. Preliminary biological data on the strongly adsorbed material indicate that the major loss in activity on recombining chromatographic eluates probably does not arise from this source (2).

Table 1. Chromatography of smoke condensate neutrals.

Eluting solvent	Silicic acid[a]		Florisil[a]	
	Activated	Non-activated	Activated	Non-activated
Petroleum ether (PE)	10.8	16.9	13.2	18.5
25% Benzene in PE	9.6	22.8	3.5	15.3
Benzene	22.6	31.9	6.8	25.5
Ether	42.4	22.9	44.1	20.1
Methanol	5.9	3.8	18.2	11.3
Total	91.3	98.3	85.8	90.7
Pyridine	.7	.3	.9	.7
Acetic acid	3.1	1.1	..	..
Total	95.1	99.7	86.7	91.4

[a] *Relative percentage neutrals removed by indicated solvent.*

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